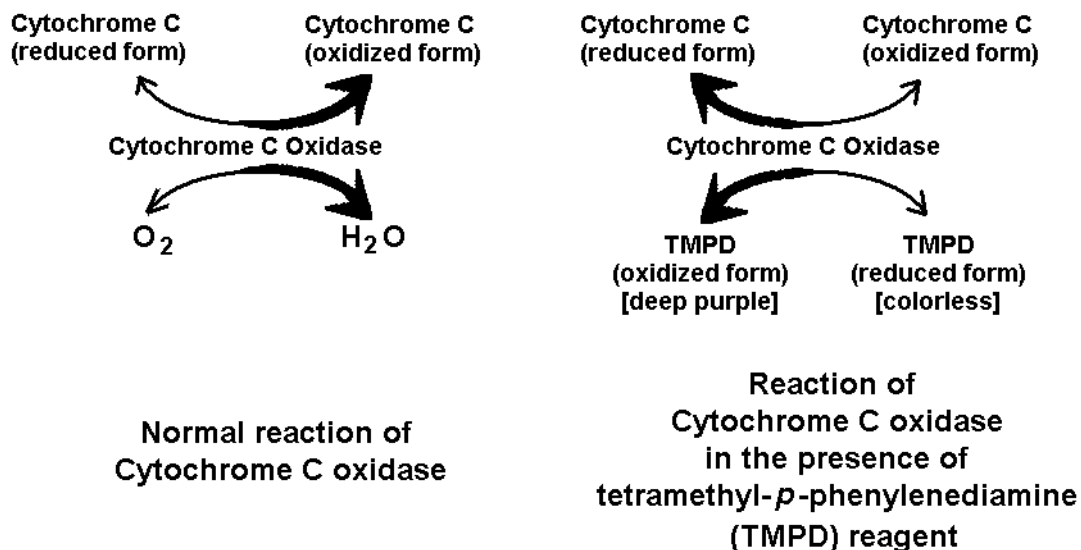


Oxidase Test

Cells conducting respiration use an electron transport chain to capture a portion of the energy found in high-energy electrons. As the electrons move through the respiratory electron transport chain, they lose energy at each step and this energy is used to drive the transport of H^+ ions from one side of the membrane to the other. Eventually the lower-energy electrons must end up on a final electron acceptor. In the case of aerobic respiration, the final electron acceptor is oxygen. Various organisms may use different electron transport pathways. The last enzyme in different electron transport pathways, that catalyzes the transfer of low-energy electrons to oxygen, can vary from one organism to the next, depending on the specific electron transport chain used by the organism (Fig. 6-74, p. 72 of the Atlas).

Organisms that use Cytochrome C Oxidase as the last electron chain enzyme, the presence of Cytochrome C Oxidase can be detected using the reagent tetramethyl-*p*-phenylenediamine (Fig. 6-75, p. 73 of the Atlas). Tetramethyl-*p*-phenylenediamine (TMPD) exists in two forms, the colorless, reduced form and the purple/blue colored, oxidized form. In the presence of Cytochrome C Oxidase (but not in the presence of other enzymes involved in transfer of low-energy electrons to oxygen), the colorless, reduced form of TMPD is oxidized to the dark purple colored, oxidized, form of TMPD as shown below.



Thus the Oxidase Test, involving the use of TMPD, detects whether an organism conducting aerobic respiration uses Cytochrome C Oxidase as the last enzyme in the electron transport chain or not. Oxidase-positive organisms (TMPD reagent turns dark purple/blue) have Cytochrome C Oxidase while Oxidase-negative organisms do not have this enzyme (TMPD remains colorless).

Note that for every oxidation (e.g. $TMPD_{[reduced\ form]}$ to $TMPD_{[oxidized\ form]}$) there must be a simultaneous reduction. In this case cytochrome C $_{[oxidized\ form]}$ is reduced to cytochrome C $_{[reduced\ form]}$. In the absence of TMPD reagent, Cytochrome C Oxidase catalyzes the normal flow of low-energy electrons from cytochrome C $_{[reduced\ form]}$ to oxygen. Remember that oxidation means *loss* of electrons and reduction means a *gain* of electrons. A molecule that is in its oxidized form has fewer electrons than a molecule that is in its reduced form.

Procedure:

To demonstrate the Oxidase Test, we will use the following test organisms:
Escherichia coli, *Pseudomonas fluorescens*, and *Alcaligenes faecalis*.

1. Obtain a strip of filter paper.
2. Add a drop of oxidase reagent solution. Be careful in handling the reagent – you should not let it touch your skin.
3. Using a sterile toothpick or wooden stick pick up a colony of the test organism and rub it over the spot of oxidase reagent. (Do not use a loop since the metal of the loop may give a false positive)
4. Dispose of the toothpick or wooden stick in the proper receptacle.
5. Observe the spot for the formation of a dark purple or blue color (oxidized form of TMPD). This should happen almost immediately, but may take up to 30 seconds. The formation of a deep purple/blue color within 30 seconds is considered a positive test. If the blue color takes a longer time to develop (e.g. after a minute), the test is considered negative.
6. Dispose of the filter strip in the proper receptacle (or repeat the test with another test organism. Add a drop of oxidase reagent to a blank spot on the paper and use a new toothpick or stick for each test you perform.)